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EXPERIMENTS ON THE DETERMINATION OF THE FATE OF THE GRAY CRESCENT MATERIAL IN THE FROG EGG.

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In the latter part of March of this year I began a series of experiments at Princeton University, having for my aim the determination of the location of the organ-forming substances, if such exist, in the cytoplasm of the frog egg. The work is scarcely more than begun and I should not ordinarily venture to present my results in such an incomplete form, but since I am leaving Princeton to enter army work it seems advisable to give a preliminary account of such results as I have obtained thus far. I hope to be able to do further experiments at a later time and to publish a full account with figures.

The method used in these experiments was to inject small amounts of a three-fourths of one per cent. aqueous solution of trypan blue, a colloidal vital (?) stain, into the cells of the early cleavage stages of the egg and into certain regions of the young blastula. Injections were made under a binocular dissecting microscope by means of exceedingly fine pointed glass pipettes in which the large upper end was sealed. By warming the upper end of the pipette part of the air would be driven out and during cooling a quantity of the stain could be sucked in. On rewarming, the stain would be forced out in a steady flow and by inserting the needle into the cell or region that I desired to inject a small amount of the stain would be left in the egg.

Since no sections have been made as yet I cannot give any report in regard to the distribution of the stain to the daughter cells in those cases where the presence of the stain or the mechan-

ical injury did not prevent further segmentation. I observed, however, that the presence of the stain induces a moribund condition which results sooner or later in the death of the cell injected or of the cells arising from it. I therefore used this method in the following experiments to kill certain regions of the egg, considering it preferable, since it can be better controlled, to the usual method of pricking with a hot needle.

Experiments with Eggs of *Rana sylvatica*.

On the morning of March 26 I brought into the laboratory a quantity of *R. sylvatica* eggs in the gray crescent stage. In nineteen of these eggs I injected one of the cells of the two-cell stage. In sixteen of the nineteen the first cleavage had come in along the median plane of bilateral symmetry, dividing the egg into right and left halves. In eight of these eggs I injected the right-hand cell and in eight the left. In one egg the first cleavage plane was oblique to the anterior-posterior axis. Whether or not the injected cell in this case contained the greater or the less amount of the gray crescent was not noted. In the other two eggs of the nineteen the first cleavage plane was transverse to the median axis, thus dividing the egg into anterior and posterior halves. In one of these two eggs I injected the anterior cell, which contained the gray crescent material, and in the other I injected the posterior cell leaving the cell containing the gray crescent material uninjured. The morning of March 28, two days after the injections had been made, I found in the egg in which the posterior cell had been killed by injection, that the anterior cell containing the gray crescent material remained uninjured and had developed into an embryo with neural folds uplifted and lying alongside the dead material resulting from the injected cell. None of the other eggs either at this time or later showed any thing resembling a neural plate. They formed a cap of live cells on a mass of dead material but developed no further.

On the morning of April 1 I collected eggs of *R. sylvatica* in the early blastula stage. The gray crescent material could still be distinguished by the characteristic pigmentation and by the smaller size of the cells there as compared with other regions

at the margin between the yolk and pigmented hemispheres. I injected the stain into ten of these blastulas, inserting my needle at right angles to the axis of the egg through the gray crescent material in such a way as to destroy nearly all of the material in that region. In eight other eggs I made a similar injection on the opposite side of the yolk field. Examination of the uninjured control eggs on the afternoon of April 3 showed them to have developed into embryos with external gills appearing. Except in two cases where there were structures which might possibly have been incomplete transverse cerebral commissures, there was no evidence of anything like a neural plate in any of the ten eggs having all or most of the gray crescent material destroyed. Closure of the blastopore was interfered with in these eggs, but apparently little if any more so than in those eggs in which the injection was made on the side of the yolk hemisphere opposite the gray crescent and from which I obtained seven neurulas out of a lot of eight eggs. The eggs with the gray crescent material destroyed were kept until the afternoon of April 4th when they were preserved, no neural plate or folds having been formed in any of them.

Experiments with Eggs of *Rana pipiens*.

The morning of April 5 I collected eggs of *R. pipiens* and brought them into the laboratory as they were passing into the two-cell stage. In forty-six of these eggs in which the first cleavage plane was anterior-posterior I injected one of the first two lateral cells. Of eight eggs in which the first cleavage plane was transverse to the anterior-posterior axis I injected the anterior cell in four eggs and the posterior cell in the other four. On account of the moribund condition resulting from injection none of the eggs injected at the two-cell stage developed far enough to give any information in regard to the fate of the gray crescent. I shall not therefore give their history here.

From eggs injected at the four-cell stage I obtained more definite results. From fourteen eggs having one of the two posterior cells of the four-cell stage injected I later obtained ten embryos defective on one side of the blastopore but having complete neural tubes.

I injected the anterior cell in four eggs in which the first two cleavage planes were oblique to the anterior-posterior axis of the egg, giving a four-cell stage with an anterior cell containing a large part of the gray crescent material, two lateral cells each containing a small amount of the gray crescent material, and a posterior cell containing none. Observation of the surface showed that though the presence of the stain did not prevent further segmentation of the injected cell, it brought about a moribund condition of the cells arising from it which prevented their taking part in development beyond the blastula stage. In three of these eggs no blastopore closure occurred though the potential blastopore was marked out by a pigmented line. In the fourth egg partial closure of the blastopore did occur and in the process the dying material, which consisted of a piece about one fourth the size of the egg, was pinched off and left free in the perivitelline space. I kept this egg under observation until the fourth day after injection but it never developed any structure that had the slightest resemblance to a neural plate though at the time of preservation it had had cilia on the surface for more than twenty-four hours and the control eggs had developed into curved embryos.

Furthermore, from eggs in which I succeeded by injection in bringing about the death of the two posterior ventral cells of the eight-cell stage I obtained a high percentage of neurulas while, on the other hand, from eggs in which I killed the two anterior ventral cells, which contained most of the gray material, I obtained no neurulas.

Further evidence that the gray crescent forms the nervous system was obtianed by making injections into young blastulas of *R. pipiens* in a manner similar to that described for *R. sylvatica*. In nine young blastulas I killed all or most of the gray crescent material by making transverse injections through the gray crescent region or by two or three radially directed injections. In five of these eggs development did not proceed beyond early gastrulation, but in four of them partial overgrowth of the yolk by the blastopore lips took place. In the process the plugs of dead material around the injected points were squeezed out of the egg into the perivitelline space. I kept these for five and

one half days after laying (until the tadpoles from the control eggs had come out of the jelly) but though they developed surface cilia and seemed to be composed of perfectly healthy tissue, they never developed any semblance of a nervous system.

I likewise destroyed the material in the region of the lower hemisphere opposite the gray crescent in twelve eggs of the same lot. Development proceeded beyond the blastula stage in only six of these but from all six of them neurulas resulted.

All of these experiments indicate that the material of the gray crescent gives rise to the neural plate.

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